P2160

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The distribution and phosphorylation of PINK1 and Parkin proteins in mice and rats.

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PINK1 (PTEN-induced putative kinase 1, a mitochondrial kinase) and Parkin (a ubiquitin ligase) play important roles in mitochondrial quality control. Parkin regulation is mediated by PINK1-dependent phosphorylation of ubiquitin. The aim of this study was to investigate the tissue distribution and phosphorylation of PINK1, Parkin proteins. We've developed a panel antibodies against the total PINK1, Parkin, phosphospecific PINK1 (pThr257), Parkin (pSer65) and the paired T257 PINK1, the paired S65 Parkin. The formalin-fixed paraffin-embedded (FFPE) tissue sections derived from BALB/c mice and Wistar rat brain and spinal cord were stained with the above antibodies by Immunohistochemistry (IHC). PINK1 and Parkin were found widely expressed in mice and rats central nervous systems (CNS), cortex neuron cells, and neurons in nucleus regions (basal nucleus, subthalamic nucleus) and the spinal cord, mainly cytoplasmic localization. In cerebellum, some Purkinje cells also showed mild expression of Parkin. The phosphospecific Parkin (pSer65) was observed in neuron cytoplasmic expression granularly, which may indicate mitochondria localization. The neuron processes and those neural tracts showed strong Parkin (pSer65) localization. PINK1 was found highly in the neuron processes and neural tracts in brain. Significant expression of PINK1 was identified in the Purkinje cell in cerebellum. The phosphospecific PINK1 were dramatically reduced in the neuron processes and neural tracts. Peripheral tissue distribution of PINK1 and Parkin showed similar patterns. Parkin was expressed in bronchial epithelial cells and type II alveolar epithelial cells in the lung and in renal tubes, and highly expressed in the neural ganglia cells in the intestine and colon. The phosphor-Parkin (pSer65) were highly expressed in the liver Kupffer cells and in kidney proximal tubules. Less expression of PINK1 was observed in the bronchial epithelial cells. PINK1 and phosphospecific PINK1 (pThr257) were mainly found in the distal tubules and collecting ducts of kidney, the neural ganglia cells in the intestine and colon. In conclusion, the PINK1 and Parkin are widely expressed in central nerve system, the phosphospecific antibodies can be useful tools for the study of PINK1/Parkin/ubiquitin signaling in Parkinson's disease.

P2161

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Investigating the roles of Mulan and Fis1 in Mitophagy.

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Mitochondria are highly dynamic organelles that respond to physiological stimuli by moving, fusing together, and dividing. Because of this high degree of interaction, it is important for cells to sequester and degrade damaged mitochondria in order to maintain the health of the mitochondrial reticulum. Though mitochondrial autophagy, or mitophagy, has traditionally been thought to require a ubiquitin ligase called Parkin, recent studies have found stress conditions that promote Parkin-independent mitophagy. These conditions may instead depend on Mulan, a ubiquitin ligase that works in parallel to Parkin in ubiquitinating mitofusin proteins, and/or Fis1, a protein that has been implicated in autophagosome recruitment during mammalian mitophagy. We investigated mitophagy rates in mouse embryonic fibroblasts under two stress conditions: the addition of the iron chelator deferiprone and post-confluent cell growth. Our results suggest that deferiprone-induced mitophagy is dependent on

Parkin, Mulan, and Fis1, while mitophagy induced in post-confluent conditions is independent of all three proteins. Both pathways rely on Atg3, a general autophagy factor. These data suggest that, in the latter condition, mitochondria are being degraded either as a by-product of increased general autophagy or through a novel mitophagy pathway. Additionally, $Fis1^{-/-}$ cells in deferiprone conditions show incomplete inactivation of a pH-sensitive mitophagy marker, which may suggest a role for Fis1 in acidifying the lysosome where mitophagy is completed.

P2162

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Regulation of Gp78 S538 phosphorylation and Gp78-dependent endoplasmic reticulum-mitochondria association and mitochondrial fission by its extracellular ligand AMF.

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Endoplasmic reticulum (ER) communication with mitochondria at membrane contact sites maintains lipid and calcium homeostasis, initiates autophagy and mitochondria genesis, and regulates cell death. Those sites are called MAMs (mitochondria-associated ER membranes) or MERCs (mitochondria-ER contacts). Autocrine motility factor receptor (AMFR/Gp78), an ER E3 ligase, has been localized to the mitochondria-associated ER and promotes ER-mitochondria association and mitochondrial fission through its E3 ubiquitin ligase activity. Both p38 MAPK dependent Gp78 S538 phosphorylation and the extracellular Gp78 ligand, autocrine motility factor/phosphoglucose isomerase (AMF), prevent Gp78 promotion of ER-mitochondria association and mitochondrial fission. However, a specific role for endogenous AMF and whether AMF acts via Gp78 S538 phosphorylation in regulation of Gp78 function at the ER-mitochondria interface has yet to be demonstrated.
First, we tested whether both exogenous and endogenous AMF regulate ER-mitochondria association. Using HT1080 fibrosarcoma cells, we found that both quenching extracellular AMF with anti-AMF antibody and inducible shAMF knockdown promoted ER-mitochondria association and mitochondrial fragmentation. Further, exogenous AMF treatment reversed the reduced ER-mitochondria association and mitochondrial fission of shAMF cells. shAMF knockdown HT1080 cells presented reduced mitochondria fluorescence intensity and while mitochondrial uncoupling with CCCP induced mitophagy in control HT-1080 cells, it did not impact mitochondria fluorescence intensity in shAMF knockdown cells.
Exogenous AMF treatment decreased binding of the 3F3A anti-Gp78 mAb (selective for dephosphorylated S538 Gp78) to immunoprecipitated FLAG-Gp78, indicating that AMF increases p38 MAPK-dependent Gp78 S538 phosphorylation. Further, in COS7 cells overexpressing FLAG-Gp78, exogenous AMF disruption of ER-mitochondria association and induction of mitochondrial elongation was prevented by Gp78 S538A mutation. This suggests that AMF regulates ER-mitochondria association through p38 MAPK-dependent Gp78 S538 phosphorylation thereby impacting Gp78 dependent ER-mitochondria association, mitochondrial fission and mitophagy.
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